

CLEAN VERSION WITH CHANGES

- 1 1. A method of isolating RNA comprising an oligo- or polynucleotide from a
2 sample comprising the steps of:
3 (a) treating the sample with a reactant capable of covalently modifying
4 the 2'-OH position of the ribose rings of the RNA under conditions
5 so that a proportion of the 2'-OH positions of the ribose rings bear a
6 substituent; and
7 (b) preparing isolated RNA therefrom by separating material containing
8 the substituent from the sample on the basis of a property of the
9 substituent.
- 1 2. The method according to claim 1, wherein:
2 (a) step (a) of Claim 1 is carried out in a reaction medium which
3 comprises an organic solvent, and optionally wherein said organic
4 solvent comprises an organic base, and further optionally wherein
5 said reactant comprises an acid anhydride, an acid chloride, a
6 carboxylic acid or an N-acylimidazole, and further optionally
7 wherein said reaction medium further comprises an acylation
8 catalyst, and further optionally wherein said the reaction medium
9 further comprises water;
10 (b) said RNA comprises mRNA, rRNA or viral RNA;
11 (c) said sample comprises a sample from a biological source;
12 (d) said sample includes DNA;
13 (e) said substituent comprises a solid phase, and optionally wherein said
14 solid phase comprises benzoyl chloride polymer bound (BCPB)
15 beads, silica particles or particles of a glass, and further optionally
16 wherein said solid phase is modified to introduce a reactive group
17 which reactive group is capable of reacting with RNA to capture the
18 RNA on the solid phase, and further optionally wherein said reactive

- 19 group is introduced by modifying the solid phase with a
20 bi-functional acid halide;
- 21 (f) said substituent comprises a hydrophobic substituent, and optionally
22 wherein said hydrophobic substituent comprises a substituent, OR,
23 wherein R is selected from the group consisting of: C₁-C₃₆ alkyl; C₁-
24 C₃₆ alkenyl; C₁-C₃₆ alkynyl; C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl;
25 C₁-C₃₆ alkoxyalkyl; C₁-C₃₆ alkylthioalkyl; C₁-C₃₆
26 alkoxyalkoxyalkyl; C₁-C₃₆ haloalkoxyalkyl; C₁-C₃₆
27 aminoalkoxyalkyl; C₆-C₃₆ aryl; C₆-C₃₆ alkylaryl; C₆-C₃₆ arylalkyl;
28 C₆-C₃₆ arylalkenyl; C₁-C₃₆ alkanoyl; C₁-C₃₆ alkenoyl; C₁-C₃₆
29 haloalkenoyl; C₁-C₃₆ haloalkanoyl; C₂-C₃₆ haloformylalkanoyl; C₁-
30 C₃₆ C₁-C₃₆ aminoalkanoyl; C₁-C₃₆ azidoalkanoyl; C₁-C₃₆
31 carboxyalkanoyl; C₁-C₃₆ carboxyalkenoyl; C₁-C₃₆ carboxyalkynoyl;
32 C₁-C₃₆ alkylaminoarylalkanoyl; C₁-C₃₆ alcoxycarbonyl; C₁-C₃₆
33 alkenyloxycarbonyl; C₁-C₃₆ alkylsulfonyl; C₆-C₃₆ arylalkanoyl; C₆-
34 C₃₆ arylalkenoyl; C₆-C₃₆ aryloxyalkanoyl; C₆-C₃₆ alkylarylalkanoyl;
35 C₆-C₃₆ haloarylalkanoyl; C₆-C₃₆ aminoarylalkanoyl; C₁-C₃₆
36 alkylsilanyl; C₁-C₃₆ trialkylsilanyl and C₁₂-C₂₈ diarylphosphano; or
37 a substituent R', wherein R' comprises C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl;
38 C₁-C₃₆ alkynyl; C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl; halo; amino;
39 C₁-C₃₆ alkylamino; C₆-C₃₆ aryl; C₁-C₃₆ alkylaryl or C₁-C₃₆ arylalkyl;
- 40 (g) said hydrophobic substituent of (f) comprises a C₄ to C₇ carbon
41 chain or ring;
- 42 (h) wherein said reactant comprises butyric anhydride, pentanoic
43 anhydride, hexanoic anhydride or benzoic anhydride;
- 44 (i) said proportion of 2'-OH positions bearing the substituent is at least
45 10%;
- 46 (j) said hydrophobic substituent of (f) comprises a C₈-C₁₂ carbon chain
47 or ring, and optionally wherein said proportion of 2'-OH positions
48 bearing the substituent is in the range 1 to 10%;

- 49 (k) said hydrophobic substituent of (f) comprises a C₁₂-C₃₆ carbon chain
50 or ring, and optionally wherein said proportion of 2'-OH positions
51 bearing the substituent is up to 1%;
52 (l) said step (b) comprises contacting the treated sample from step (a)
53 with a hydrophobic solid phase so as to bind the material containing
54 the hydrophobic substituent and optionally washing the material
55 bound to the solid phase, and optionally wherein said hydrophobic
56 solid phase comprises hydrophobic particles, and further optionally
57 wherein said method further comprises a step of eluting the material
58 bound to the hydrophobic solid phase by treating with a detergent, a
59 chaotropic or a solvent, by lowering the salt concentration or by
60 cleaving the substituent from the 2'-OH position of the ribose rings;
61 (m) said step (b) comprises the further step of treating the treated sample
62 from step (a) with a lyotropic salt to aggregate the material
63 containing the hydrophobic substituent as an RNA precipitate, and
64 isolating the precipitate, and optionally wherein said lyotropic salt
65 comprises ammonium sulphate, an alkali metal chloride, magnesium
66 chloride or calcium chloride; or
67 (n) said step (b) comprises treating the treated sample with a non-polar
68 solvent to form a hydrophobic liquid phase which contains the
69 material containing the hydrophobic substituent, and isolating the
70 hydrophobic liquid phase, and optionally wherein said non-polar
71 solvent comprises pentane, cyclohexane, toluene, benzene, light
72 petroleum, xylene or hexane.

1 3. A kit for the preparative isolation of RNA comprising an oligo- or
2 polynucleotide from a sample, which kit comprises:

- 3 (i) a reaction system for modifying the RNA to form a modified oligo-
4 or poly-nucleotide in which a proportion of the 2'-OH positions of
5 the ribose rings bear a substituent; and

6 (ii) a separation system for preparing isolated RNA by separating
7 material containing the substituent from the sample on the basis of a
8 property of the substituent.

1 4. The kit according to Claim 3, wherein said reaction system comprises:
2 (a) an organic solvent; and
3 (b) a reactant capable of covalently modifying the 2'-OH position of the
4 ribose rings of the RNA in the presence of the organic solvent, and
5 optionally wherein:
6 (i) said organic solvent comprises an organic base;
7 (ii) said reactant comprises an acid anhydride, an acid chloride, a
8 carboxylic acid or an N-acylimidazole;
9 (iii) said kit comprises an acylation catalyst;
10 (iv) said substituent comprises a solid phase, and optionally
11 wherein said solid phase comprises benzoyl chloride
12 polymer bound (BCPB) beads, silica particles or particles of
13 a glass; and optionally wherein:
14 (c) said substituent comprises a hydrophobic substituent, or more
15 specifically wherein said hydrophobic substituent comprises a
16 substituent, OR, wherein R comprises a moiety selected from the
17 group consisting of: C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl; C₁-C₃₆ alkynyl;
18 C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl; C₁-C₃₆ alkoxyalkyl; C₁-C₃₆
19 alkylthioalkyl; C₁-C₃₆ alkoxyalkoxyalkyl; C₁-C₃₆ haloalkoxyalkyl;
20 C₁-C₃₆ aminoalkoxyalkyl; C₆-C₃₆ aryl; C₆-C₃₆ alkylaryl; C₆-C₃₆
21 arylalkyl; C₆-C₃₆ arylalkenyl; C₁-C₃₆ alkanoyl; C₁-C₃₆ alkenoyl; C₁-
22 C₃₆ haloalkenoyl; C₁-C₃₆ haloalkanoyl; C₂-C₃₆ haloformylalkanoyl;
23 C₁-C₃₆ C₁-C₃₆ aminoalkanoyl; C₁-C₃₆ azidoalkanoyl; C₁-C₃₆
24 carboxyalkanoyl; C₁-C₃₆ carboxyalkenoyl; C₁-C₃₆ carboxyalkynoyl;
25 C₁-C₃₆ alkylaminoarylalkanoyl; C₁-C₃₆ alkoxycarbonyl; C₁-C₃₆
26 alkenyloxycarbonyl; C₁-C₃₆ alkylsulfonyl; C₆-C₃₆ arylalkanoyl; C₆-
27 C₃₆ arylalkenoyl; C₆-C₃₆ aryloxyalkanoyl; C₆-C₃₆ alkylarylkano

24 (j) said separation system comprises a non-polar solvent for forming a
25 hydrophobic liquid phase which contains the material containing the
26 hydrophobic substituent.

1 6. A preparative device for isolating RNA comprising an oligo- or
2 polynucleotide from a sample from a subject, which device comprises:
3 (i) a means for extracting the sample from the subject;
4 (ii) a reaction system for modifying RNA in the sample to form a
5 modified oligo- or poly-nucleotide in which a proportion of the 2'-
6 OH positions of the ribose rings bear a substituent; and
7 (iii) a separation system for preparing isolated RNA by separating material
8 containing the substituent from the sample on the basis of a property
9 of the substituent.

1 7. The device according to claim 6, wherein:
2 (a) said means for extracting the sample from the subject comprises a
3 syringe needle;
4 (b) said substituent comprises a solid phase, and optionally wherein the
5 solid phase comprises a membrane, a particle, a bead, a filter, a
6 fibre, a gel, a strip, a matrix, a resin, a capillary or the walls of a
7 vessel;
8 (c) said sample comprises biological material; or
9 (d) said device further comprises a filter for removing red and/or white
10 blood cells.

28 C₆-C₃₆ haloarylalkanoyl; C₆-C₃₆ aminoarylalkanoyl; C₁-C₃₆
29 alkylsilanyl; C₁-C₃₆ trialkylsilanyl and C₁₂-C₂₈ diarylphosphano; or
30 a substituent R', wherein R' comprises C₁-C₃₆ alkyl; C₁-C₃₆ alkenyl;
31 C₁-C₃₆ alkynyl; C₁-C₃₆ haloalkyl; C₁-C₃₆ aminoalkyl; halo; amino;
32 C₁-C₃₆ alkylamino; C₆-C₃₆ aryl; C₁-C₃₆ alkylaryl or C₁-C₃₆ arylalkyl.
33

1 5. The kit according to claim 4, wherein:

- 2 (a) said hydrophobic substituent comprises a C₄ to C₇ carbon chain or
3 ring;
4 (b) said reactant comprises butyric anhydride, pentanoic anhydride,
5 hexanoic anhydride or benzoic anhydride;
6 (c) said proportion of 2'-OH positions bearing the substituent is at least
7 10%;
8 (d) said hydrophobic substituent comprises a C₈-C₁₂ carbon chain or
9 ring;
10 (e) said proportion of 2'-OH positions bearing the substituent is
11 selected from any one integer from 1 to 10% inclusive;
12 (f) said hydrophobic substituent comprises a C₁₂-C₃₆ carbon chain or
13 ring;
14 (g) said proportion of 2'-OH positions bearing the substituent is up to
15 1%;
16 (h) said separation system comprises a hydrophobic solid phase for
17 binding the material containing the substituent, and optionally
18 wherein said hydrophobic solid phase comprises hydrophobic
19 particles, and further optionally wherein said separation system
20 further comprises an elution medium for eluting RNA bound to the
21 hydrophobic solid phase;
22 (i) said separation system comprises a lyotropic salt for aggregating
23 the material containing the hydrophobic substituent; or